Genetic Recombination at the Buff Spore Color Locus in SORDARIA BREVICOLLIS. II. Analysis of Flanking Marker Behavior in Crosses between Buff Mutants

GENETIC RECOMBINATION AT THE BUFF SPORE COLOR LOCUS IN *SORDARIA BREVICOLLIS*. II. ANALYSIS OF FLANKING MARKER BEHAVIOR IN CROSSES BETWEEN BUFF MUTANTS

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ABSTRACT

Aberrant asci containing one or more wild-type spores were selected from crosses between pairs of alleles of the buff locus in the presence of closely linked flanking markers. Data were obtained relating to the site of aberrant segregation and the position of any associated crossover giving recombination of flanking markers. Aberrant segregation at a proximal site within the buff gene may be associated with a crossover proximal to the site of aberrant segregation or, with equal frequency, with a crossover distal to the site of the second mutant present in the cross. Similarly, segregation at a distal site may be associated with a crossover distal to the site or, with lower frequency, with a crossover proximal to the site of the proximal mutant present in the cross. Crossovers between the alleles were rare. This evidence for the relationship between hybrid DNA and crossing over is discussed in terms of current models for the mechanism of recombination.

CURRENT models of the mechanism of genetic recombination consider gene conversion and crossing over to be consequences of the same primary event. The MESELSON and RADDING (1975) model proposes that hybrid DNA formation is initiated asymmetrically outside a gene by single-strand transfer between paired homologues. Hybrid DNA forms on one chromatid and moves into the gene. This can lead to postmeiotic segregation and conversion at any nonhomologous sites included in the heteroduplex. Strand isomerization can take place after dissociation of the enzyme to give recombinant flanking markers if the two crossed strands are cut. The hybrid DNA may move through the gene by rotary diffusion, generating symmetrical hybrid DNA as in the HOLLIDAY (1964) model. Cutting of equivalent pairs of crossed strands will give parental or recombinant chromatid arms, depending on whether or not isomerization has occurred.

The MESELSON and RADDING model predicts a gradient of asymmetric events, decreasing from the site of initiation of hybrid DNA, and a corresponding increase in symmetric events. This prediction is supported by the data from the

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b2 locus of *Ascobolus immersus* obtained by PAQUETTE and ROSSIGNOL (1978) and ROSSIGNOL, PAQUETTE and NICOLAS (1978). The model also has implications for the position of crossovers in relation to hybrid DNA. Crossovers are predicted to occur at the distal end of the hybrid DNA, if its formation is initiated on the proximal side of the gene, and, conversely, at the proximal end, if the event is initiated on the distal side of the gene. Hybrid DNA is continuous between the site of initiation and a crossover.

The relationship between gene conversion and crossing over can be investigated to some extent by analyzing outside marker behavior associated with gene conversion at a linked site. More information can be obtained by a detailed study of interallelic recombination. Interallelic recombination in fungi is not usually caused by classical crossing over between two alleles. It is a result of nonreciprocal events. FOGEL and HURST (1967) studied flanking marker behavior in relation to gene conversion at the histidine-1 locus of *Saccharomyces cerevisiae*. SAVAGE and HASTINGS (1981) obtained further data from this locus. Comparable results for the *grey* gene of *Sordaria fimicola* are given by KITANI and WHITEHOUSE (1974).

We describe here results from two-point crosses at the buff locus of *Sordaria brevicollis* with outside markers present. SANG and WHITEHOUSE (1979) described the results of crosses between buff mutants and wild type using the same flanking markers. We concluded that hybrid DNA formation giving aberrant segregation at buff was mainly asymmetric. The frequency of recombination associated with aberrant segregation was significantly less than 50%. The recombination frequency was lower for odd-ratio aberrant asci than for even-ratio asci. The study described here was carried out to analyze the relationship between aberrant segregation and crossing over in more detail. These results are compared with those from the similar studies. The implications for models of the mechanism of recombination are discussed.

MATERIALS AND METHODS

(a) Mutants: The buff gene is located about 4 map units from the centromere in the right arm of linkage group II of *S. brevicollis*. The buff mutants used were three ultraviolet-induced mutants, *YS9*, *C47* and *C67*, and one ethylmethanesulfonate-induced mutant, *YS132*. Analysis of these mutants in one-point crosses has been described by SANG and WHITEHOUSE (1979). Each mutant has a characteristic conversion pattern. *C47*, *C67* and *YS132* give both postmeiotic segregation and conversion aberrant asci in crosses to wild type. Throughout this paper where ratios of wild-type and mutant spores are given the number of wild-type spores is written first and the number of mutant second, e.g., six wild type: two mutant is given as 6:2. *YS9* gives mainly 6:2 aberrant asci. *C47* is the most proximal mutant, and *C67* is the most distal, according to our results and those reported by MACDONALD and WHITEHOUSE (1979). The criteria for mapping the alleles were the relative frequencies of the two recombinant flanking marker genotypes in wild-type recombinants from pairwise crosses of the mutants in *trans*. The relative positions of *YS132* and *YS9* are uncertain. The flanking markers used were *met-1*, a methionine-requiring mutant, and *nic-1*, a nicotinamide-requiring mutant described by MACDONALD and WHITEHOUSE (1979) and BOND (1973), respectively. The *met-1* locus is located 5 units to the left of buff and, therefore, is about 1 unit from the centromere in the left arm of the chromosome. The *nic-1* locus is 2 units to the right of buff. In *trans* crosses postmeiotic segregation and conversion occur more frequently at distal than proximal sites with respect to the centromere, except with pairs of mutants mapping near the proximal end of the gene (BOND 1973; MACDONALD and WHITEHOUSE 1979).

(b) Culture media: Crossing, germination and minimal media were as defined by FIELDS and OLIVE (1967). Minimal medium supplemented with 0.01 g/liter of nicotinamide or methionine was used to test for the auxotrophic markers.

(c) Method: Crosses were made between pairs of buff mutants and cultured at 30° for 8 days. The general cross was *met-1* 1 + + × + + 2 *nic-1*. In all crosses analyzed C67 was the distal (2) allele. C67 is darker than the other mutants and has a higher germination frequency. This effect is not autonomous. All spores from a cross between C67 and another buff allele show increased germination. This is a great advantage in analyzing asci from two-point crosses as poor germination would make complete octad analysis difficult.

Asci containing one or more wild-type spores were selected from squash preparations of ascal clusters in 8% glucose solution. The spores were dissected out and allowed to germinate at 37° for approximately 6 hr. The resulting single-spore cultures were tested to determine the flanking marker configuration and backcrossed to both buff mutant parents to identify the buff alleles present. Only a limited number of buff spores were scored for the buff alleles present.

Two studies were undertaken. The first was to ascertain the position of the crossover giving flanking-marker recombination, as far as possible, in relation to aberrant segregation within the buff gene. The second set of experiments was done to give an estimate of the extent of hybrid DNA in buff, particularly to find if both alleles or only one were included in hybrid DNA. In the first set of experiments the buff allele was identified only in buff spores with marker recombination. In the second set of experiments only 1:7 asci were analyzed, but the genotype of the buff sister spore to the single wild-type spore was determined for all asci.

RESULTS

I. Classification of asci: In the first experiment the asci were classified in terms of 1:7 or 2:6 segregation, the allele that showed aberrant segregation and flanking marker configuration. The chromatids involved in any aberrant segregation or crossover events and the locations of crossovers were also determined. Possible crossover locations are shown in Figure 1.

For 1:7 asci the position of the crossover can be localized, as at either x or z (Figure 1), provided the hybrid DNA is confined to one chromatid. The need for hybrid DNA to be asymmetric is illustrated in Table 1 where 5:3 segregation at

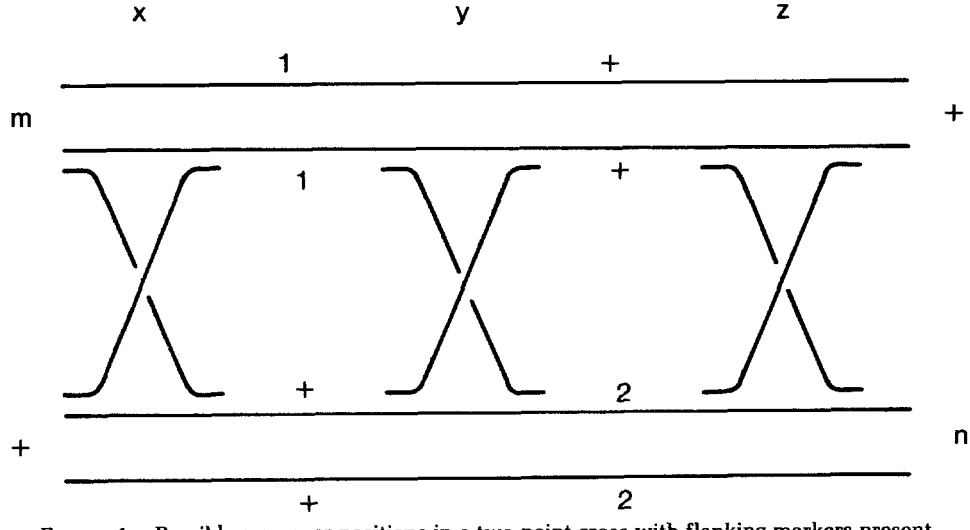

FIGURE 1.—Possible crossover positions in a two-point cross with flanking markers present.

TABLE 1

Crossover localization with asymmetric or symmetric hybrid DNA formation

	Parental			Position of crossover (Figure 1)							
				x				y			
(A)											
m	*1*	+	+	*m*	*1*	+	+	*m*	*1*	+	+
m	*1/+*	+	+	*m*	+	*2*	*n*	*m*	*1/+*	*2*	*n*
+	+	*2*	*n*	+	*1/+*	+	+	+	+	+	+
+	+	*2*	*n*	+	+	*2*	*n*	+	+	*2*	*n*
	Tritype										
(B)											
m	*1*	+	+	*m*	*1*	+	+	*m*	*1*	+	+
m	+	+	+	*m*	*1/+*	*2*	*n*	*m*	+	*2*	*n*
+	*1/+*	*2*	*n*	+	+	+	+	+	*1/+*	+	+
+	+	*2*	*n*	+	+	*2*	*n*	+	+	*2*	*n*
	Tetratype										

Ascus genotypes that result from crossing over adjacent to a mutant showing 5:3 segregation when a second allele shows normal 4:4 segregation. In (A) the hybrid DNA could be confined to one chromatid (asymmetric), but in (B) it involves both (symmetric).

the proximal site is considered. If the aberrant segregation results from hybrid DNA in one chromatid only, two of the four products of meiosis will have the same genotype in a noncrossover ascus, giving a tritype ascus (Table 1). The corresponding genotypes with a crossover at x or y (Figure 1) are shown in the table. If the 5:3 segregation results from hybrid DNA in both chromatids, there will have been mismatch correction to wild type in one chromatid but not the other. Alternatives (A) and (B) in Table 1 will result, depending on which chromatid showed the correction. In (B) the noncrossover genotype has all four products of meiosis differing in genotype. The corresponding crossover genotypes are the same as those found in (A) with x and y transposed. If hybrid DNA is symmetric, alternatives (A) and (B) will be expected with equal frequency. It will not be possible to discriminate between crossing over at x or y. In the buff gene the hybrid DNA seems to be predominantly asymmetric. In one-point crosses aberrant 4:4 asci were very rare. Only 3% of the odd-ratio postmeiotic segregation asci noncrossover for flanking markers were tetratype, implying 6% symmetric hybrid DNA formation (SANG and WHITEHOUSE 1979).

It is not possible to locate the crossover as precisely in 2+:6*m* asci: recombinant asci with aberrant segregation at 1 may have an adjacent crossover at x or y. Similarly, in asci with aberrant segregation at 2 the crossover may be at y or z.

The basis for the classification used for the results of the first experiments is given in Table 2A and B. The scheme shown is for postmeiotic segregation at either the proximal or distal allele. Conversion asci can be classified in a similar manner. Table 2A considers asci with postmeiotic segregation at the proximal allele, and Table 2B considers asci with postmeiotic segregation at the distal allele. The influence of incidental crossovers on three basic classes of asci is considered. Class I are aberrant asci with parental flanking markers. Class II

are asci with an aberrant segregation at one allele and an adjacent crossover of outside markers. Class III contains an unexpected class of ascus. In these asci the crossover appears not to be adjacent to the allele showing aberrant segregation, *i.e.*, an aberrant segregation at 1 associated with a crossover at z or an aberrant segregation at 2 with a crossover at x.

Classes IV and V are class I asci with proximal or distal incidental crossovers. Classes VI to XI include class II and III asci in which incidental crossovers have occurred. The classes P, Q, R and S in Table 2 were not recognized because not all buff spores were tested for the buff allele present. P asci would have been scored as I, Q asci as IV or V, R asci as II and S asci as III, in each case with reference to Table 2A or B. These classes would be expected to be infrequent because classes VI to IX, arising in comparable ways, were rare.

Small numbers of asci that did not fit this classification were found. Five asci had a crossover at *y*, between the two buff alleles, giving recombinant chromatids of the genotype *m 1 2 n*/+ + + +. These reciprocal recombinant asci all showed outside marker recombination. Reciprocal recombinants not recombined for flanking markers would have been included in class I. Fourteen other asci that could not be classified as described were found and will be shown separately (see section IV).

The data from the second set of experiments, in which 1:7 asci were analyzed completely, were classified as outlined in Table 3. This analysis was possible only if the genotype of the buff sister spore of the single wild-type spore was known. These asci were classified in terms of the minimum extent of the hybrid DNA that could have given the different ascus genotypes. The hybrid DNA could have included only one allele or both. The two possibilities are shown in Figure 2. In (i) or (ii) hybrid DNA includes one allele. Only one mismatch is produced which, if correction does not occur, will give a 1:7 ascus. In (iii) the hybrid DNA extends through both alleles giving two mismatches. If the +/2 mismatch is corrected to give +/+, the resulting 1:7 ascus will be indistinguishable from a 1:7 ascus resulting from a single-site event, as in (i). if the *1*/+ mismatch is corrected to +/+, the resulting ascus will show a 6:2 segregation at *1* and a 3:5 segregation at *2*. This class of ascus indicates that hybrid DNA has spanned both alleles in a two-point cross.

These asci are included in classes B and D in Table 3. The frequency of asci in classes B and D can be used to estimate the frequency of single-site events. It is assumed that all mutants show conversion to wild type or mutant with equal frequency. This appears to be justified because the mutants used in the second set of experiments (*C47*, *YS132* and *C67*) show approximate parity in conversion to wild type and to mutant (SANG and WHITEHOUSE 1979; MACDONALD and WHITEHOUSE 1979). If hybrid DNA extends to both sites the numbers in classes A and B will be expected to be equal. Similarly, the numbers in classes C and D will be expected to be equal. If hybrid DNA is confined to one site only, only class A or class C asci would be expected, the R2 genotype requiring a detached crossover. An estimate of the frequency of single-site events is given by

$$\frac{(A + C) - (B + D)}{A + B + C + D}.$$

TABLE 2

Influence of incidental crossovers on parental and recombinant flanking marker configurations

Event	Proximal or distal	Incidental crossover: Strand relationship			
		2 strand	3 strand	3 strand	4 strand
A. 1:7 asci with postmeiotic segregation at the proximal site					
		m 1 + +	m 1 + +	m 1 + +	m 1 + +
		+ 1/+ + +	+ 1/+ + +	+ 1/+ + +	+ 1/+ + +
m 1 + +	Proximal	m + 2 n	+ + 2 n	+ + 2 n	m + 2 n
m 1/+ + +		+ + 2 n	m + 2 n	m + 2 n	+ + 2 n
+ + 2 n		II	II	IV	IV
+ + 2 n					
I		m 1 + +	m 1 + +	+ 1 + n	+ 1 + n
Parental		m 1/+ + n	m 1/+ + n	+ 1/+ + +	+ 1/+ + +
	Distal	+ + 2 +	+ + 2 n	m + 2 +	m + 2 n
		+ + 2 n	+ + 2 +	m + 2 n	m + 2 +
		III	III	V	V
		m 1 + +	m 1 + +	m 1 + +	m 1 + +
		+ + 2 n	+ + 2 n	+ + 2 n	+ + 2 n
m 1 + +	Proximal	m 1/+ + +	+ 1/+ + +	+ 1/+ + +	m 1/+ + +
m + 2 n		+ + 2 n	m + 2 n	m + 2 n	+ + 2 n
+ 1/+ + +		I	II	IV	VI
+ + 2 n					
II		m 1 + +	m 1 + +	+ 1 + n	+ 1 + n
Proximal crossover		m + 2 +	m + 2 n	+ + 2 +	+ + 2 n
	Distal	+ 1/+ + n	+ 1/+ + n	m 1/+ + +	m 1/+ + +
		+ + 2 n	+ + 2 +	m + 2 n	m + 2 +
		P	Q	R	VII
		m 1 + +	m 1 + +	m 1 + +	m 1 + +
		+ 1/+ + n	+ 1/+ + n	+ 1/+ + n	+ 1/+ + n
m 1 + +	Proximal	m + 2 +	+ + 2 +	+ + 2 +	m + 2 +
m 1/+ + n		+ + 2 n	m + 2 n	m + 2 n	+ + 2 n
+ + 2 +		P	Q	S	VIII
+ + 2 n					
III		m 1 + +	m 1 + +	+ 1 + n	+ 1 + n
Distal crossover		m 1/+ + +	m 1/+ + n	+ 1/+ + +	+ 1/+ + n
	Distal	+ + 2 n	+ + 2 n	m + 2 +	m + 2 +
		+ + 2 n	+ + 2 +	m + 2 n	m + 2 +
		I	III	V	IX

B. 1:7 asci with postmeiotic segregation at the distal site

					m	*1*	+	+	*m*	*1*	+	+	+	*1*	+	+	+	*1*	+	+
					+	*1*	+	+	+	*1*	+	+	*m*	*1*	+	+	*m*	*1*	+	+
m	*1*	+	+	Proximal	*m*	+	+/2	*n*	+	+	+/2	*n*	*m*	+	+/2	*n*	+	+	+/2	*n*
m	*1*	+	+		+	+	*2*	*n*	*m*	+	*2*	*n*	+	+	*2*	*n*	*m*	+	*2*	*n*
+	+	+/2	*n*				III				IV				III				IV	
+	+	*2*	*n*																	
	I				*m*	*1*	+	+	*m*	*1*	+	+	*m*	*1*	+	*n*	*m*	*1*	+	*n*
	Parental				*m*	*1*	+	*n*	*m*	*1*	+	*n*	*m*	*1*	+	+	*m*	*1*	+	+
				Distal	+	+	+/2	+	+	+	+/2	*n*	+	+	+/2	+	+	+	+/2	*n*
					+	+	*2*	*n*	+	+	*2*	+	+	+	*2*	*n*	+	+	*2*	+
							II				V				II				V	
					m	*1*	+	+	*m*	*1*	+	+	+	*1*	+	+	+	*1*	+	+
					+	*1*	+	*n*	+	*1*	+	*n*	*m*	*1*	+	*n*	*m*	*1*	+	*n*
m	*1*	+	+	Proximal	*m*	+	+/2	+	+	+	+/2	+	*m*	+	+/2	+	+	+	+/2	+
m	*1*	+	*n*		+	+	*2*	*n*	*m*	+	*2*	*n*	+	+	*2*	*n*	*m*	+	*2*	*n*
+	+	+/2	+				P				R				Q				VI	
+	+	*2*	*n*																	
	II				*m*	*1*	+	+	*m*	*1*	+	+	*m*	*1*	+	*n*	*m*	*1*	+	*n*
	Distal crossover				*m*	*1*	+	+	*m*	*1*	+	*n*	*m*	*1*	+	+	*m*	*1*	+	*n*
				Distal	+	+	+/2	*n*	+	+	+/2	*n*	+	+	+/2	+	+	+	+/2	+
					+	+	*2*	*n*	+	+	*2*	+	+	+	*2*	*n*	+	+	*2*	+
							I				V				II				VII	
					m	*1*	+	+	*m*	*1*	+	+	+	*1*	+	+	+	*1*	+	+
					+	+	+/2	*n*	+	+	+/2	*n*	*m*	+	+/2	*n*	*m*	+	+/2	*n*
m	*1*	+	+	Proximal	*m*	*1*	+	+	+	*1*	+	+	*m*	*1*	+	+	+	*1*	+	+
m	+	+/2	*n*		+	+	*2*	*n*	*m*	+	*2*	*n*	+	+	*2*	*n*	*m*	+	*2*	*n*
+	*1*	+	+				I				IV				III				VIII	
+	+	*2*	*n*																	
	III				*m*	*1*	+	+	*m*	*1*	+	+	*m*	*1*	+	*n*	*m*	*1*	+	*n*
	Proximal crossover				*m*	+	+/2	+	*m*	+	+/2	*n*	*m*	+	+/2	+	*m*	+	+/2	*n*
				Distal	+	*1*	+	*n*	+	*1*	+	*n*	+	*1*	+	+	+	*1*	+	+
					+	+	*2*	*n*	+	+	*2*	+	+	+	*2*	*n*	+	+	*2*	+
							P				S				Q				IX	

TABLE 3

Genotypes expected for 1:7 asci from a two-point cross, if hybrid DNA is confined to one chromatid

Class	Minimum distribution of hybrid DNA	Site with post-meiotic segregation	Flanking marker genotype of wild-type spore											
			P1 (A, B) P2 (C, D)				R1				R2			
A	One site	Proximal	*m*	*1*	+	+	*m*	*1*	+	+	*m*	*1*	+	+
			m	*1/+*	+	+	*m*	+	*2*	*n*	*m*	*1/+*	+	*n*
			+	+	*2*	*n*	+	*1/+*	+	+	+	+	*2*	+
			+	+	*2*	*n*	+	+	*2*	*n*	+	+	*2*	*n*
B	Both sites	Distal	*m*	*1*	+	+	*m*	*1*	+	+	*m*	*1*	+	+
			m	+	*+/2*	+	*m*	+	*2*	*n*	*m*	+	*+/2*	*n*
			+	+	*2*	*n*	+	+	*+/2*	+	+	+	*2*	+
			+	+	*2*	*n*	+	+	*2*	*n*	+	+	*2*	*n*
C	One site	Distal	*m*	*1*	+	+	*m*	*1*	+	+	*m*	*1*	+	+
			m	*1*	+	+	*m*	*1*	+	*n*	*m*	+	*+/2*	*n*
			+	+	*+/2*	*n*	+	+	*+/2*	+	+	*1*	+	+
			+	+	*2*	*n*	+	+	*2*	*n*	+	+	*2*	*n*
D	Both sites	Proximal	*m*	*1*	+	+	*m*	*1*	+	+	*m*	*1*	+	+
			m	*1*	+	+	*m*	*1*	+	*n*	*m*	*1/+*	+	*n*
			+	*1/+*	+	*n*	+	*1/+*	+	+	+	*1*	+	+
			+	+	*2*	*n*	+	+	*2*	*n*	+	+	*2*	*n*

Parents: $\frac{m \quad 1 \quad + \quad +}{+ \quad + \quad 2 \quad n}$

P1 = *m* + (parental combination, originally associated with proximal allele, 1)
P2 = + *n* (parental combination, originally associated with distal allele, 2)
R1 = + +
R2 = *m* *n*

II. Data from first experiments: In the first experiments a total of 304 aberrant asci was analyzed from the three crosses carried out. Two hundred and eighty-five of these asci could be classified as in Table 2 A and B. These results are given in Table 4A–C. Where two numbers are given for a particular class the first is the number of asci definitely in that class. The second is the number of asci possibly in that class but where incidental crossovers could not be scored because of insufficient germination. In the following calculations this distinction is ignored. The number of asci that cannot be completely classified is small in relation to the distribution of asci between the classes. For example, in Table 4A 1:7 class IB is given as 16 + 3, *i.e.*, 16 definitely IB and 3 either IB, IVB or VB. Only two asci were found in 1:7 IVB and VB. The three incompletely scored asci were, therefore, likely to have been in class IB. Similar conclusions were reached in all cases.

The identification of the incidental crossovers enables a correction to be applied to the actual number of crossover asci, II + III. This allows for the occurrence of undetected crossovers between the markers but not related to the aberrant segregation.

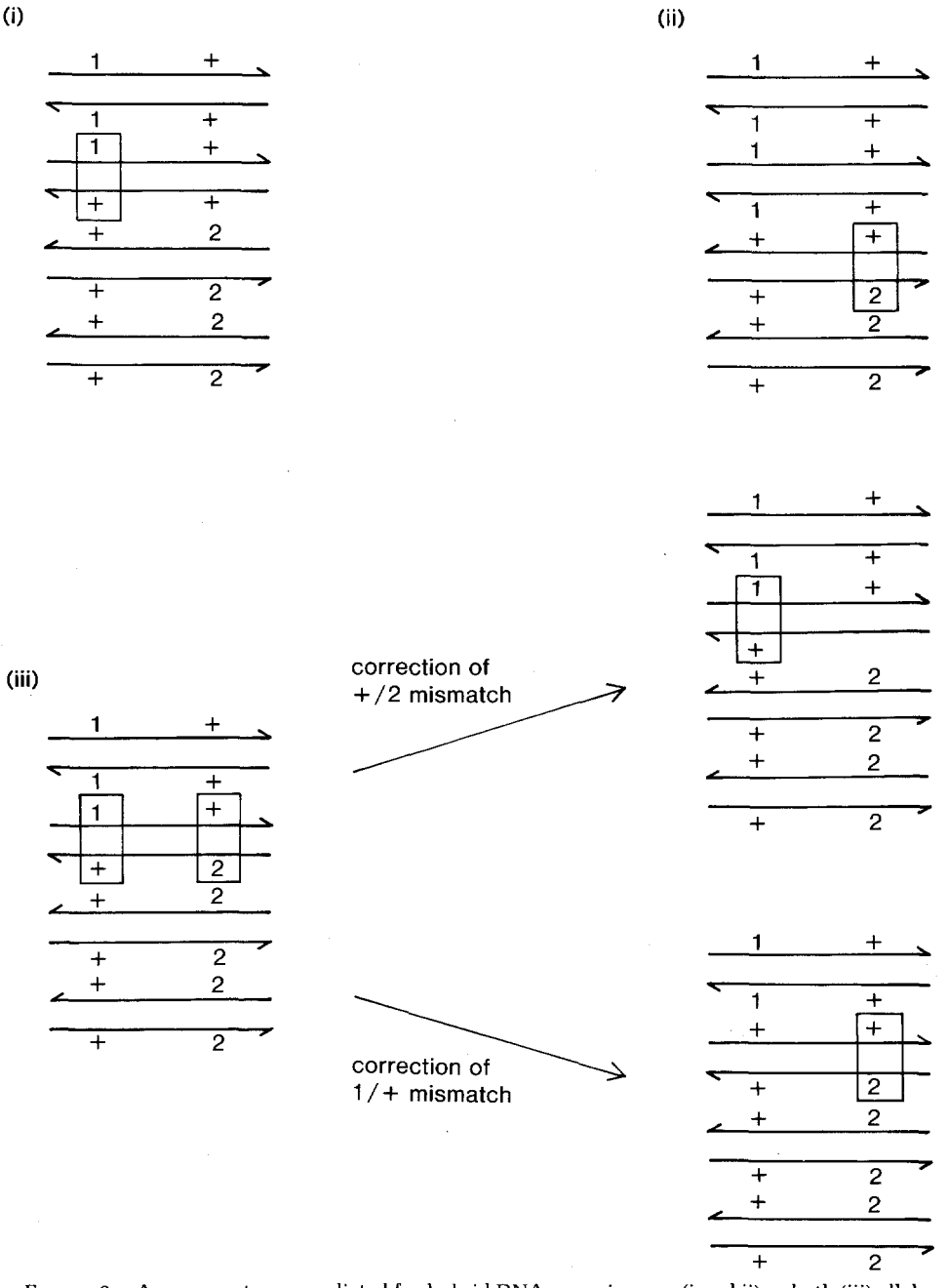

FIGURE 2.—Ascus genotypes predicted for hybrid DNA spanning one (i and ii) or both (iii) alleles in a two-point cross. Rectangles show mispaired sites.

One quarter of the incidental crossovers with asci in classes II and III will be detected, assuming no chromatid interference, because they will give rise to asci in classes VI to XI. Only two such asci were found; therefore the occurrence of

TABLE 4

1:7 and 2:6 asci

Cross				Class	1:7 A	1:7 B	1:7 A or B	1:7 Total	2:6 A	2:6 B	2:6 A or B	2:6 Total	Total
A				I	10	16 + 3		29	9	12		21	50
				II	4	6	1	11	2	7	3	12	23
met-1	*C47*	+	+	III	3			3	2		1	3	6
				IV		1		1	1			1	2
+	+	C67	*nic-1*	V		1		1	2			2	3
A:C47 B:C67				Total	17	27	1	45	16	19	4	39	84
				Class									
B				I	5 + 1	10		16	24 + 5	5		34	50
				II			1	1	11	4	4	19	20
met-1	*YS9*	+	+	III	1	1		2	11		1 + 1	13	15
				IV		1		1	2			2	3
+	+	C67	*nic-1*	V					1	1		2	2
A:YS9 B:C67				VI/VII							1	1	1
				Total	7	12	1	20	54	10	7	71	91
				Class									
C				I	8 + 2	18 + 1		29	23	9		32	61
				II	2	3		5	11	9	5 + 1	26	31
met-1	*YS132*	+	+	III	3	1		4	8	3	1	12	16
				IV/V	1			1				1	
+	+	C67	*nic-1*	VIII					1			1	1
A:YS132 B:C67				Total	16	23		39	43	21	7	71	110

There were no asci in classes VI–IX in A, VIII and IX in B and VI, VII and IX in C.

other undetected crossovers was ignored. Asci from classes VI and VII were added to II, and asci from VIII and IX were added to III.

Asci in classes IV and V all derive from I. It was assumed that similar numbers were incorporated in classes II and III, but that these really belonged in I. For aberrant segregations at the proximal site the contribution to II is expected to be equal to IV and to III to equal V. For aberrant segregations at the distal site the contribution to II will equal that to V, and to III that to IV.

Table 5 gives an analysis of the results from the three crosses considered. The frequency of recombination of outside markers was calculated. The data were corrected, and the corrected frequency was calculated. The proportion of crossovers that were nonadjacent was determined, *i.e.*, the percentage of class III asci in the total number of crossover asci. From Table 5 the following conclusions can be drawn.

(1) The outside marker recombination frequency associated with aberrant segregation at buff is 36%. Approximately one-third of the crossovers of outside markers were not adjacent to the allele showing aberrant segregation.

(2) The frequency of outside marker recombination associated with aberrant segregation at buff is an average of the frequency found with 1:7 and the frequency with 2:6 segregation. Twenty-one percent of the 1:7 asci showed associated marker recombination, whereas 44% of the 2:6 asci were recombinant. This difference is significant: $\chi^2 = 15.26$, $n = 1$, $P < 0.001$. Approximately one-third of the associated crossovers were nonadjacent.

(3) The frequency of outside marker recombination associated with aberrant segregation at *1* is approximately equal to that at *2*. There is a difference in the number of nonadjacent crossovers associated with aberrant segregation at the proximal allele and the number associated with aberrant segregation at the distal allele. Almost one-half of the crossovers associated with aberrant segregation at *1* are nonadjacent, whereas only 10% are at *2*. This difference is significant: $\chi^2 = 12.79$, $n = 1$, $P < 0.001$.

III. Reciprocal recombinant asci: Five reciprocal recombinant asci were found, representing less than 2% of the total number of asci analyzed. They were distributed as follows: two from the *C47* cross, one from the *YS9* cross and two from the *YS132* cross.

IV. Other aberrant asci: Fourteen asci, or 4% of the total asci analyzed, could not be classified as in Table 2 or as reciprocal recombinants. These are shown in Table 6. Some of these asci, e.g., the *a* type, could be the result of asymmetric hybrid DNA spanning both buff alleles. Several, *c*, *d*, *e* and *f*, could result from symmetric hybrid DNA spanning both sites, accompanied by correction of some of the mismatches produced. These asci could also have resulted from two asymmetric events.

V. Analysis of 1:7 asci: Fifty-three 1+:7m asci from the cross *met-1 C47* × *C67 nic-1* and 22 from the cross *met-1 YS132* × *C67 nic-1* were analyzed and classified as in Table 3. Any asci that could not be unambiguously classified as in a, b, c or d are not included in the calculations. The results are given in Table 7A and B.

These data were used to calculate the extent of hybrid DNA within the buff gene giving rise to aberrant segregations. It was assumed that hybrid DNA

TABLE 5

Summary of results of analysis of recombinant asci from the cross

$$\frac{met\text{-}1 \quad 1 \quad + \quad +}{+ \quad + \quad 2 \quad nic\text{-}1}$$

No.	Class of aberrant ascus	Uncorrected values			Corrected values					
		Non-crossover	Crossover	% crossover	Non-crossover	Crossover	% crossover	±S.E.	% III / II + III	±S.E.
1	Total asci	172	113	40	183	102	36	3	32	5
2	1+:7*m* asci	78	26	25	82	22	21	4	32	10
	2+:6*m* asci	94	87	48	101	80	44	4	32	5
3	Asci with aberrant segregation at proximal allele	94	59	39	101	52	34	4	49	7
	Asci with aberrant segregation at distal allele	78	34	30	82	30	27	4	10	5
4	Asci with 1+:7*m* segregation at proximal allele	27	13	33	28	12	30	7	54	14
	Asci with 1+:7*m* segregation at distal allele	51	11	18	54	8	13	4	0	0
5	Asci with 2+:6*m* segregation at proximal allele	67	46	41	73	40	35	5	48	8
	Asci with 2+:6*m* segregation at distal allele	27	23	46	28	22	44	8	14	7

In some asci the allele showing aberrant segregation could not be determined. These asci are not included in 3, 4 and 5 of this table.

formation was asymmetric. From Table 7A the estimated frequency of single-site events for the *C47* × *C67* cross is:

$$\frac{(A + C) - (B + D)}{A + B + C + D} = \frac{48 - 2}{50} = 92\%.$$

From Table 7B the estimated frequency of single-site events for the *YS132* × *C67* cross is:

$$\frac{19 - 2}{21} = 81\%.$$

These estimates do not differ significantly, the mean value being 63/71 = 89%.

TABLE 6

Rare aberrant asci from cross $\frac{met\text{-}1 \quad 1 \quad + \quad +}{+ \quad + \quad 2 \quad nic\text{-}1}$

Aberrant segregation +:m	Genotype				Minimum no. of chromatids involved in event	Cross: C47 × C67	Cross: YS9 × C67	Cross: YS132 × C67
a 1:7	m	*1*	+	+	2	0	1	1
	m	+	+/2	*n*				
	+	+	*2*	+				
	+	+	*2*	*n*				
b 1:7	m	*1*	+	+	2	0	1	0
	m	*1*/+	+/2	*n*				
	+	+	*2*	+				
	+	+	*2*	*n*				
c 1 + 1:6	m	*1*	+	+	2	1	0	0
	m	*1*/+	+	+				
	+	+	+/2	*n*				
	+	+	*2*	*n*				
d 3:5	m	*1*	+	+	2	0	1	0
	m	+	+	+				
	+	+	+/2	*n*				
	+	+	*2*	*n*				
e 4:4	m	*1*	+	+	2	0	3	0
	m	+	+	+				
	+	+	+	*n*				
	+	+	*2*	*n*				
f 4:4	m	*1*	+	+	2	0	0	1
	m	+	+	*n*				
	+	+	+	+				
	+	+	*2*	*n*				
g 4:4	m	+	+	+	3	1	0	0
	m	*1*	+	*n*				
	+	+	+	+				
	+	+	*2*	*n*				
h 4:4	m	+	+	+	3	0	2	1
	m	+	+	+				
	+	+	*2*	*n*				
	+	+	*2*	*n*				
i 6:2	m	*1*	+	+	3	0	1	0
	m	+	+	*n*				
	+	+	+	+				
	+	+	+	*n*				

TABLE 7

Genotypes of 1:7 asci

Class	Minimum distribution of hybrid DNA	Site with postmeiotic segregation	Flanking marker genotype of wild-type spore: *P1* (A, B) *P2* (C, D)	*R1*	*R2*
		A. From the cross *met-1* *C47* / + + × + + / *C67* *nic-1*			
A	One site	Proximal	8	0	3
			1		
B	Both sites	Distal	0	0	0
C	One site	Distal	30	1	3
			1	2	1
D	Both sites	Proximal	1	1	0
		B. From the cross *met-1* *YS132* / + + × + + / *C67* *nic-1*			
A	One site	Proximal	7	1	0
			1		
B	Both sites	Distal	1	1	0
C	One site	Distal	4	4	2
D	Both sites	Proximal	0	0	0

DISCUSSION

These data, from the analysis of aberrant asci from crosses between two alleles of the buff locus with outside markers present, provide information on the location of crossovers in relation to sites of gene conversion. From this a relationship between hybrid DNA and crossover positions in recombination can be inferred. In summary, aberrant segregation at a proximal site in the buff gene may be associated with a crossover proximal to the site of aberrant segregation or, with equal frequency, with a crossover distal to the second mutant present in the cross. Similarly, aberrant segregation at a distal site may be associated with a crossover distal to the site or, with lower frequency, with a crossover proximal to the proximal mutant present in the cross. Median crossovers, between the alleles, were rare. These conclusions are based on two assumptions. The first is that hybrid DNA formation was predominantly asymmetric. The second is that the hybrid DNA involved in producing an aberrant segregation only rarely included both mutants present in the cross. Hybrid DNA appeared to involve the second site in only 11% of cases, whereas about 50% of crossovers in this second experiment were not adjacent to the mutant showing aberrant segregation.

In the MESELSON and RADDING and other models of recombination hybrid DNA formation is proposed to be initiated outside a gene. Hybrid DNA extends into a gene for varying distances. This is suggested as an explanation of polarity observed in gene conversion (MURRAY 1963). The hybrid DNA is then resolved by cutting pairs of DNA strands to give either parental or recombinant marker combinations. The MESELSON and RADDING model predicts that crossovers will frequently occur within a gene, after a region of hybrid DNA. Our results indicate that crossovers rarely occur between the sites of the alleles. In about 50% of cases they occur in the interval between the allele that shows aberrant segregation and the flanking marker on that side of the gene. This implies that the crossover position might be near the site of initiation of hybrid DNA formation. Unexpectedly, a proportion, as much as 50%, of crossovers appears to be nonadjacent, separated from the mutant showing gene conversion by a region not included in hybrid DNA. Hybrid DNA may not be continuous between the site of aberrant segregation and the location of the crossover. This observation is not consistent with the MESELSON and RADDING model.

If the sites of the buff mutants have been wrongly mapped and need to be transposed, the effect would be to transpose the adjacent and nonadjacent crossover classes. Crossovers proximal to the site of aberrant segregation become crossovers distal to the second mutant site, and *vice versa*. Since neither class of crossover is predicted by the MESELSON and RADDING model, the problem of their origin remains.

KITANI and OLIVE (1969) and KITANI and WHITEHOUSE (1974) have obtained comparable data from interallelic crosses of the *grey* gene in *S. fimicola*. When hybrid DNA was asymmetric, median crossovers were rare (see WHITEHOUSE 1982). In most asci the crossover appears to have been near the probable site of initiation of hybrid DNA formation. These asci were approximately 20 times more frequent than those with a median crossover.

The data of FOGEL and HURST (1967) and SAVAGE and HASTINGS (1981), who analyzed two-point crosses of mutants of the *his-1* locus of *Saccharomyces cerevisiae*, provide further evidence for nonadjacent crossovers. Approximately 25% of crossovers associated with conversion of the proximal mutant were distal to the second mutant present in the cross. At the *grey* locus of *S. fimicola*, 30% of crossovers associated with postmeiotic segregation at a distal allele were on the proximal side of the gene. These crossovers were not incidental to the aberrant segregation since they involved the same chromatids. Undetected incidental crossovers can be allowed for, as in this study, and do not substantially alter the data. MACDONALD and WHITEHOUSE (1979) also found a high frequency of single-site events associated with nonadjacent crossovers for the *YS17* buff mutant.

Two possible mechanisms that could give rise to nonadjacent crossovers are illustrated in Figure 3. In Figure 3(i) the explanation favored by SAVAGE and HASTINGS (1981) for their results is illustrated. Hybrid DNA is initiated proximal to the first mutant, *1*, and spreads through both mutants *1* and *2*. The crossed strands are then resolved to give a crossover distal to *2*. The mismatch produced at *1* is corrected to wild type to give conversion of *1*, whereas the mismatch at *2* is corrected to wild type, which restores a normal 4:4 segregation at *2*. There

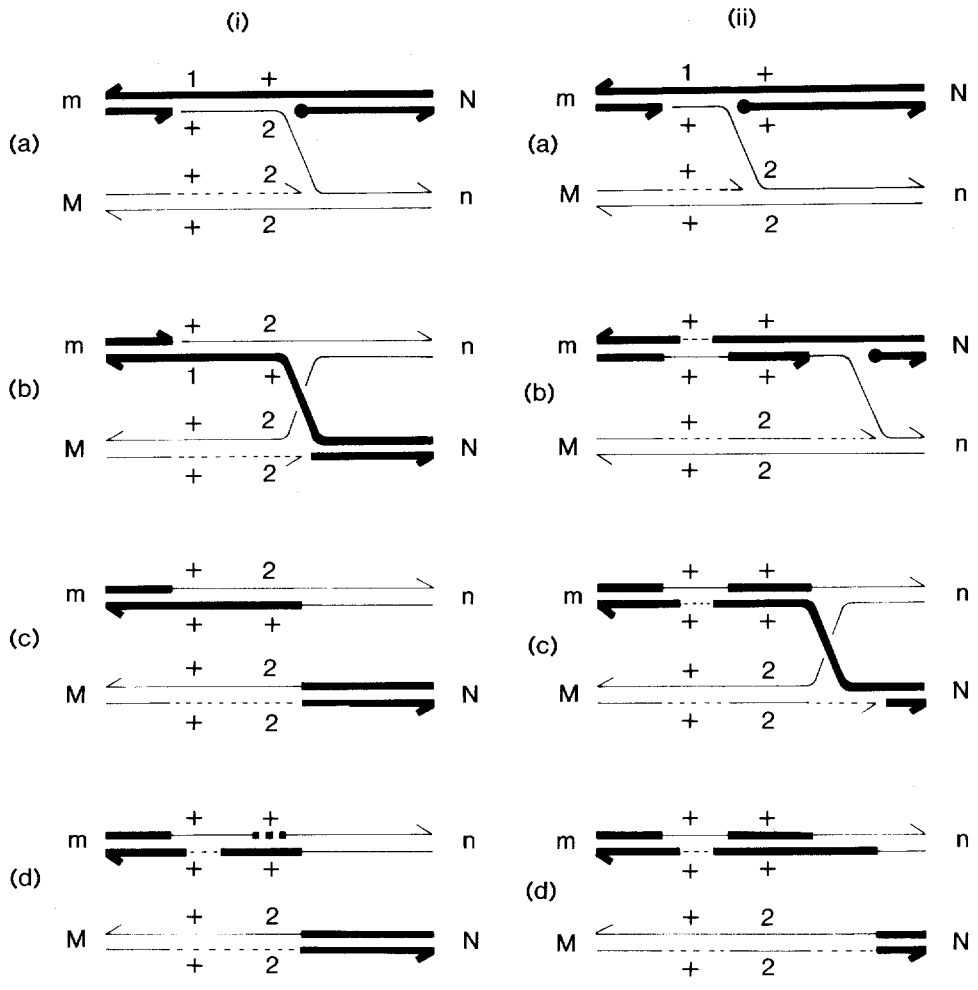

FIGURE 3.—Two possible mechanisms that could give rise to nonadjacent crossovers, based on the MESELSON and RADDING model. M/m and N/n are flanking markers. 1 and 2 are two mutants within a gene. (i) *a*: Transfer of a single DNA strand between paired homologues as the initial event in recombination. Asymmetric hybrid DNA spreads through the gene including both 1 and 2, creating two mismatches. *b*: Isomerization takes place giving two crossed strands. *c*: Crossed strands are cut to give recombination of chromatid arms. *d*: Mismatch correction to give conversion of 1 and restore normal 4:4 segregation at 2. (ii) *a*: Single-strand transfer of DNA initiated to the left of the gene and including only mutant 1. *b*: Correction of the +/1 mismatch to +/+ giving conversion of 1. Single-crossing strand is cut before isomerization has occurred, with the result that crossing over cannot take place. Second initiation event to right of both mutants 1 and 2. *c*: Isomerization to give a HOLLIDAY structure. *d*: Resolution of the HOLLIDAY structure to give recombination of the distal flanking markers associated with gene conversion at 1. This figure has been reprinted with the permission of John Wiley and Sons, Ltd., from *Genetic Recombination. Understanding the Mechanisms*, by H. L. K. WHITEHOUSE (1982).

will be no evidence that 2 was included in the hybrid DNA. Evidence for restoration of normal 4:4 segregation by mismatch correction is given by data from the b2 locus of *A. immersus* discussed by ROSSIGNOL and HAEDENS (1978).

Our results for the buff locus suggest that, in nonadjacent crossover asci, the

aberrant segregation and crossover could be separate, although associated, events. The normal 4:4 segregation at the second site could be a primary event and not the result of restoration correction. Figure 3(ii) illustrates a possible mechanism involving two separate events. Here hybrid DNA formation is initiated proximal to 1 and is terminated between 1 and 2, leaving the chromatids in parental configuration. Hybrid DNA is then initiated at a second site, distal to 2, and this time isomerization takes place, and the outside markers are recombined.

FINCHAM (1974) has previously suggested that in some cases at least two separate events are involved in gene conversion and crossing over. He proposed this as an explanation for his results for the *am-1* gene of *Neurospora crassa*. In pairwise crosses of *am-1* mutants in the presence of flanking markers one of the noncrossover genotypes, P1 (see Table 3), was more frequent among random prototrophs than the other, P2, in almost every cross. This could be accounted for by initiation of recombination being predominantly on the proximal side of the gene. This explanation was not consistent with the frequencies of the two recombinant genotypes among the prototrophs. R1 and R2 (equivalent to our class III, nonadjacent recombinant asci) were almost equal in frequency. This observation of P1 > P2 but R1 = R2 raised the question of how the R2 genotype arose. FINCHAM (1974) proposed that R1 and R2 resulted, predominantly at least, from hybrid DNA extending to one allelic site only, but that crossovers were often initiated separately nearby. If this initiation occurred on the opposite side of the gene from the event that gave rise to conversion, then an *R2* genotype would result.

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